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Antihypertensive activity of *Zingiber officinale* and *Korean ginseng* in experimentally induced hypertension in rats

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SUMMARY

We investigated the antihypertensive effect of Pet ether extract (PE) of ginger rhizome; its toluene fraction (TF) and *Korean ginseng* extract (KGE) in deoxycorticosterone acetate (DOCA) – salt induced and fructose induced hypertensive rats. In DOCA model, DOCA (25 mg/kg, once a week; s.c) was administered in uninephrectomised animals for 4 w. PE (50 mg/kg/day; p.o), TF (10 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o) were evaluated for their antihypertensive effect. In the fructose model, drinking water was replaced with fructose (10%) for five weeks to induce hypertension. PE (50 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o) were assessed for its antihypertensive effect in fructose model. After completion of the treatment schedule, vascular reactivity to various agonists like 5-HT, noradrenaline, adrenaline, phenylbiguanide and acetylcholine were recorded in rats of both the models. A cumulative dose response curve (CDRC) of 5-HT was carried out in isolated rat fundus strip of the fructose induced hypertensive rats. Chronic administration of PE (50 mg/kg/day; p.o), TF (10 mg/kg/day; p.o), and KGE (30 mg/kg/day; p.o) significantly reduced the blood pressure in DOCA salt whereas PE (50 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o) reduced the blood pressure in fructose induced hypertensive rats. Treatment with PE (50 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o) in fructose model for five weeks shifted the CDRC towards the right on rat fundus. The mechanism of action may partly involve the serotonergic antagonistic property.

Key words: Deoxycorticosteroneacetate; Ginger; Ginseng; 5-Hydroxytryptamine; Hypertension; Fructose

INTRODUCTION

The rhizome part of *Zingiber officinale* (Zingiberaceae) commonly known as ginger is widely used in the traditional system of medicine. Ginger possesses antiemetic (Yamahara *et al*., 1989), antiplatelet (Srivastava 1984; Lumb, 1994) anti-inflammatory and antipyretic (Sharma *et al*., 1994), antiulcer (Yamahara *et al*., 1998), antioxidant (Reddy and Lokesh, 1992), antihepatotoxic (Hikino *et al*., 1985), antiinfective properties (Denyer *et al*., 1994) and blood pressure lowering effect (Ghayur *et al*., 2005; Ghayur and Gilani, 2005). Ginger is also useful for migraine headaches (Mustafa and Srivastava, 1990). Ginger also possesses carminative, laxative, expectorant (Kirtikar and Basu, 1993), hypocholesterolaemic

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(Giri, 1984), and hypoglycaemic activity (Sharma and Shukla, 1977). Ginger contains gingerols and galanolactone, both having antagonistic activity at 5-HT_3 receptor (Huang *et al*., 1991). The 5-HT_3 receptor antagonistic properties of ginger accounts for its anti-emetic effects (Bone *et al*., 1990; Fischer *et al*., 1991). In search for alternatives to antihypertensive drugs, the 5-HT_3 receptor antagonists are currently being considered for their potential use in hypertension (Tsukomoto *et al*., 2000). *Korean (Panax) ginseng* (Araliaceae) has a blood pressure lowering effect (Han *et al*., 1998; Jeon *et al*., 2000; Stavro *et al*., 2004), antistress and anabolic activity (Grandhi *et al*., 1994) and improves learning and memory (Sung-Ha Jui *et al*., 1999). It contains triterpene glycosides named ginsenosides which account for the medicinal action of the root of the plant. At least 13 ginsenosides have been identified falling into 2 groups based upon the aglycone portion: protopanaxodiol (diols) and protopanaxatriol (triols). They are classified according to an alphanumeric system i.e. Ra, Rb, Rb2, Rc, etc. The plant also contains sterols, acetylenic compounds and peptidoglycans named Panaxans (Wren, 1988; Mills, 1991).

Serotonin (5-HT) is a naturally occurring vasoactive substance that has diverse cardiovascular effects. These effects can be explained by the existence of 5-HT receptor subtypes, which mediate different biological actions. The vasoconstrictive actions of 5-HT are mediated by 5-HT_2 serotonergic receptors, and 5-HT also amplifies the release and activities of other vasoconstrictors such as angiotensin and norepinephrine. Abnormalities in the serotonergic system may play an important role in the pathophysiology of multiple cardiovascular disease states such as systemic hypertension, primary pulmonary hypertension and peripheral vascular disease (Frishman and Grewall, 2000). 5-HT exhibits complex actions on cardiovascular system (Kuhn *et al*., 1980). Its vasodilator effect is thought to be mediated by 5-HT_{1A} receptor located on sympathetic nerves or in the blood vessel wall. It causes vasodilation partly by releasing nitric oxide from endothelial cells (Cohen and Vanhoutte, 1985) and partly by inhibiting noradrenaline release from sympathetic nerve terminals. 5-HT3 receptors are unique among the families of 5-HT receptors, in that they are nonselective Na+/K+ ion channel receptors (ligand operated ion channel). They are found in the parasympathetic terminals of the gastrointestinal tract and also in the central nervous system, particularly in the area postrema, nucleus tractus solitarii, frontal cortex, and hippocampus. Preclinical studies suggest that 5-HT3 antagonists may enhance memory and be of benefit in the treatment of anxiety, depression, pain, and dementia. 5-HT3 antagonists have proven clinically effective for the treatment of chemotherapy-induced or radiation-induced nausea and vomiting (Hardman and Limbard, 2001).

Selye and Bois were the first to demonstrate that deoxycorticosterone acetate (DOCA) produces hypertension in rats (Selye and Bois, 1957). The hypertensive mechanism due to chronic administration of DOCA-salt has been well documented. There is increased DOCA induced reabsorbtion of salt and water leading to increased blood volume and hence increased blood pressure. It has been hypothesized that 5-HT_{2B} receptor is upregulated and necessary for maintaining elevated blood pressure (BP) in rats made hypertensive by DOCA and N-nitro-L- arginine methyl ester (NAME) (nitric oxide synthase inhibitor) (Banes and Watts, 2003). Physiologically this up regulation is important because 5-HT has a 300-fold greater affinity for 5-HT_{2B} receptor compared to 5-HT_{2A} receptor (Wainscott *et al*., 1993). Fructose is widely present in numerous foods. It has been commonly used as a sweetner and promoted as being useful for weight reduction, exercise endurance and diabetes (Dai and McNeill, 1995). It has been demonstrated that hypertension develops when normal rats are fed with fructose enriched diet (Verma *et al*., 1994; Suzuki *et al*., 1997). Ginger and ginseng has not been evaluated much in both the types of hypertensive models. The objective of our present

study was to investigate the antihypertensive activity of petroleum ether extract and toluene fraction of ginger and *Korean ginseng* extract in DOCA- salt and fructose induced hypertensive models in rats with an attempt to explore the possible mode of action.

MATERIALS AND METHODS

Preparation of extract

One kg of dried rhizome of *Zingiber officinale* (Zingiberaceae) was purchased from commercial sources. The rhizomes were authenticated and finely powdered. It was defatted with petroleum ether (60 - 80°C) by maceration with agitation process. The constituents of the petroleum ether extract were separated by column chromatography using toluene (Evans, 2002). The petroleum ether extract (PE) and its toluene fraction (TF) were used for the studies. The PE gave a yield of 3.38% w/w, and TF gave a yield of 0.5% w/w. The chemical tests indicated the presence of terpenes (Harborne, 1973).

The *Korean ginseng* extract (KGE) i.e *Panax ginseng extract* was obtained as a gift sample from Glenmark Pharmaceuticals, Nashik. It was manufactured by Pangin Biotech Co. Ltd, Korea. The standardized and controlled ginseng slender tail roots was extracted 3 times under 70°C for about 8 h in the extraction apparatus with 70% of ethanol. The extract was concentrated in vacuo at a reduced pressure of 500 - 600 mmHg under 60 - 70°C till the ginseng extract was obtained. It contained 18% w/w of saponins.

Animals

Wistar rats of either sex (40 female and 20 male weighing 200 - 250 g) were housed in plastic cages in groups of five under standard lab conditions of temp 25 ± 1°C with 12 h light/dark cycle. Animals had free access to standard chow diet and water. For arterial blood pressure measurements using the tail cuff method (non-invasive method), animals were trained at least one week until the blood pressure was steadily recorded with minimal stress and restraint. The first cardiovascular parameters were discarded and the mean of five or six subsequent measurements were recorded once a week. The Institutional Animal Ethical Committee approved the protocol of the study.

Drugs and chemicals

Noradrenaline (Nor), Adrenaline (Adr), 5-hydroxytryptamine (5-HT), Acetylcholine (ACh), Phenylbiguanide (PBG), urethane and deoxycorticosterone acetate (DOCA) were purchased from Sigma (Sigma Chemicals, St. Louis, USA). Fructose was purchased from SD Fine Chemicals. DOCA was dispersed in cottonseed oil. Dilutions of PE, TF and KGE were prepared in fresh distilled water containing Tween 80 (less than 0.5% of the total volume) and administered orally. Tween 80 was used as a solubilizing agent as PE and TF were insoluble in water. All drug solutions were freshly prepared in distilled water before each experiment. 10% Fructose solution was freshly prepared in tap water. Nor, Adr, 5-HT and ACh were dissolved in fresh distilled water whereas PBG was solubilized using Tween 80 (less than 0.5% of the total volume). Petroleum ether and toluene were purchased from local sources.

Induction of hypertension

DOCA salt induced hypertension:

Hypertension was induced experimentally in female Wistar rats (200 - 250 g) by unilateral nephrectomy (Nagawa and Nasjletti, 1988). Rats were anaesthetized with ether. A lateral incision was made in the area overlying the kidney. The renal blood vessel was ligated with fine sterile silk thread and the kidney was removed. The incision was sutured and closed with Michel clips. All operated rats received an injection of ampicillin (10 mg/kg, i.p) daily for 5 days. Neosporin powder (Polymixin B sulfate BP, Zinc bacitracin BP, neomycin sulfate IP) was applied locally to prevent infection. One week later DOCA

(25 mg/kg, once a week; s.c; for 4 w) dispersed in cottonseed oil was injected to uninephrectomised rats. 1% saline + 0.2% KCl *ad libitum* was given instead of drinking water. In sham operated control animals, a similar procedure was performed except the treatment of DOCA.

Fructose induced hypertension:

Hypertension was induced experimentally in male Wistar rats (200 - 250 g) by giving 10% fructose solution to drink *ad libitum* for five weeks. Fructose solution was prepared every two days by dissolving the fructose in tap water. Ordinary tap water was given to control animals to drink throughout the whole experimental period (Vogel, 2002a).

Experimental protocol

DOCA-salt induced hypertension model:

Forty unilateral nephrectomized female animals were randomized and housed into 8 groups of 5 animals each.

Group 1: Sham Control: Sham operated control rats received 0.2 ml. of cottonseed oil; s.c.; once a week; for 4 w. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 2: PE-50: Unilateral nephrectomized rats received PE (50 mg/kg/day; p.o.) and cottonseed oil (0.2 ml/rat/once a week; s.c.) for 4 w. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 3: TF-10: Unilateral nephrectomized rats received TF (10 mg/kg/day; p.o.) and cottonseed oil (0.2 ml/rat/once a week; s.c.) for 4 w. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 4: KGE-30: Unilateral nephrectomized rats received KGE (30 mg/kg/day; p.o.) and cottonseed oil (0.2 ml/rat/once a week; s.c.) for 4 w. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 5: DOCA: Unilateral nephrectomized rats received DOCA-salt, (25 mg/kg/once a week; s.c.) dispersed in cottonseed oil, for 4 w. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 6: DOCA+PE-50: Unilateral nephrectomized rats received DOCA-salt (25 mg/kg/once a week; s.c.) dispersed in cottonseed oil and PE (50 mg/kg/day, p.o.) for 4 weeks. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 7: DOCA+TF-10: Unilateral nephrectomized rats received DOCA-salt (25 mg/kg/once a week; s.c.) and TF (10 mg/kg/day; p.o.) for 4 w. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 8: DOCA+KGE-30: Unilateral nephrectomized rats received DOCA-salt (25 mg/kg/once a week; s.c.) and KGE (30 mg/kg/day; p.o.) and cottonseed oil (0.2 ml/rat/twice a week; s.c.) for 4 w. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Fructose induced hypertension model:

Twenty male Wistar rats (200 - 250 g) were randomized and divided into 4 groups of 5 each.

Group 1: Control: Animals received no medication, but given tap water for drinking.

Group 2: F-10: Animals received 10% fructose solution instead of drinking water, *ad libitum* for 5 w.

Group 3: F-10 + PE-50: Animals received 10% fructose solution instead of drinking water, *ad libitum,* with PE (50 mg/kg/day, p.o.) for 5 w.

Group 4: F- 10 + KGE-30: Animals received 10% fructose solution instead of drinking water, *ad libitum,* with KGE (30 mg/kg/day, p.o.) for 5 w.

Measurement of blood pressure

Measurement of Blood Pressure by noninvasive (indirect) method:

Systolic blood pressure was measured weekly for five weeks by indirect non invasive tail cuff method using Letica 5,002 Storage Pressure Meter (PANLAB BLOOD PRESSURE RECORDER model LE 2002 N, Italy).

Measurement of Blood Pressure by invasive (direct) method:

After completion of treatment schedule rats from

each group were anesthetized with urethane (120 mg/100 g). Femoral vein was cannulated with fine polyethylene catheter for administration of drug. Tracheostomy was performed and blood pressure was recorded from left common carotid artery using pressure transducer by direct method on BIOPAC Data Acquisition System (BIOPAC MP30 SYSTEM, USA) (Vogel, 2002b). Heparinised saline (250 IU/mL) was filled in the transducer and in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting. After 30 min of stabilization, blood pressure and vascular reactivity to Adr (0.5 g/kg), Nor (0.5 g/kg), 5-HT (0.5 g/kg), PBG (0.5 g/kg) and ACh (0.5 g/kg) were recorded.

***In- vitro* studies**

After completion of treatment schedule in fructose induced hypertension model, rats from each group were sacrificed by stunning, fundus was removed and placed in Krebs solution. A strip of fundus was mounted in a bath containing Krebs solution. The physiologic salt solution had the following composition (mM) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2 and Glucose, 11. The physiologic salt solution had a pH of 7.4. It was warmed to 37°C and aerated with 95% O_2 and 5% CO_2 (Carbogen). One end was tied to an aerator tube and the other end to a forced displacement transducer (Ugo Basile, Comerio Italy). Each strip was placed under optimum resting tension (1.5 g) and allowed to equilibrate for 30 min with frequent changes of Krebs solution at 10 min interval. Contractile response to each dose of 5-HT was recorded for 90 sec for each tissue preparation on a Gemini Two Channel Recorder 7,070 (Ugo Basile) (Goyal, 1999).

Gas chromatography mass spectroscopy

TF was dissolved in chloroform and subjected to Gas chromatography mass Spectroscopy (Perkin Elmer, USA) and the mass spectrum was obtained (Mendham, 2002).

Statistics

All data are shown as mean ± S.E.M. Statistical analysis was performed with one-way ANOVA followed by Dunnett's test. Differences of $P < 0.05$ was considered statistically significant.

RESULTS

Antihypertensive effect of PE-50, TF-10 and KGE-30 on DOCA-salt hypertensive rats

Administration of PE-50, TF-10 and KGE-30 in DOCA salt unilateral nephrectomised rats significantly ($P < 0.05$) reduced the mean arterial blood pressure at the end of the 2nd, 3rd week and showed further reduction at the end of the 4th week as compared to DOCA salt hypertensive rats alone, implying an antihypertensive effect of PE, TF and KGE (Fig. 1).

Effect of PE-50, TF-10, and KGE -30 on vascular reactivity to Adr, Nor, 5-HT and PBG in DOCA-salt hypertensive rats:

Pressor responses to Nor, Adr and 5-HT were not altered significantly in case of PE-50, TF-10, and

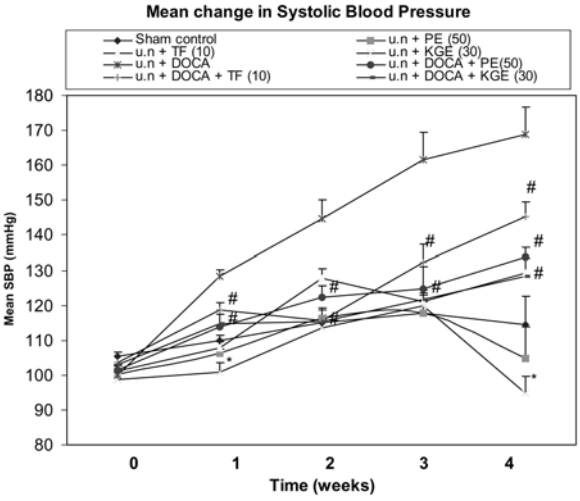

Fig. 1. Time course (weekly) of changes in mean arterial pressure (mmHg) during 4 w in Sham control, PE-50, TF-10, KGE- 30, DOCA, DOCA + PE-50, DOCA + TF-10, DOCA + KGE-30 treated groups. $^{*}P < 0.05$ when compared to sham control group and $^{\#}P < 0.05$ when compared to DOCA hypertensive rats. Vertical lines represent S.E.M. (n = 5). SBP: Systolic Blood Pressure.

KGE -30 treated rats as compared to sham control rats. Pressor responses to Nor, Adr were significantly ($P < 0.05$) increased in case of uninephrectomised DOCA-salt treated hypertensive rats as compared to sham control rats. Pressor responses to 5-HT was significantly ($P < 0.05$) reduced in case of uninephrectomised rats which received PE-50, TF-10 and KGE-30 compared to sham control and in uninephrectomised DOCA-salt treated rats which received PE-50, TF-10, and KGE-30 for 4 w as compared to DOCA-salt hypertensive rats. Pressor responses to PBG was significantly ($P < 0.05$) reduced in case of uninephrectomised animals receiving PE-50 and TF-10 for 4 weeks as compared to sham control and in uninephrectomised DOCA-salt treated rats which received PE-50 and TF-10 for 4 w as compared to DOCA-salt hypertensive rats (Figs. 2-5).

Antihypertensive effect of PE-50 and KGE-30 in fructose induced hypertensive rats

Fructose (10% solution) feeding for 5 w in rats produced a significant ($P < 0.05$) elevation of SBP (171.5 ± 6.82 mmHg) as compared to control rats (124.7 ± 3.69 mmHg). However chronic administration of PE-50 and KGE-30 for 5 w in fructose fed rats significantly ($P < 0.05$) reduced SBP (132.71 ± 14.03 mmHg, 138.5 ± 10.5 mmHg) as compared to fructose fed hypertensive rats (171.5 ± 6.82 mmHg) implying an antihypertensive effect (Fig. 6).

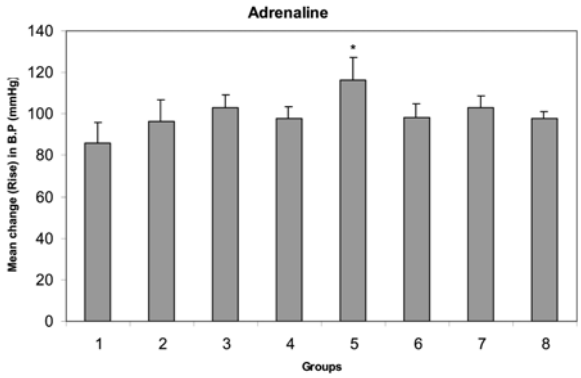

Fig. 2. Mean change in blood pressure to adrenaline (0.5 g/kg) in (1)-Sham control, (2)-PE-50, (3)-TF-10, (4)-KGE- 30,(5)- DOCA, (6)-DOCA +PE-50, (7)-DOCA + TF-10, (8)-DOCA + KGE-30 treated groups. $^{*}P < 0.05$ when compared to sham control. Vertical lines represent S.E.M. (n = 5). B.P: Blood Pressure.

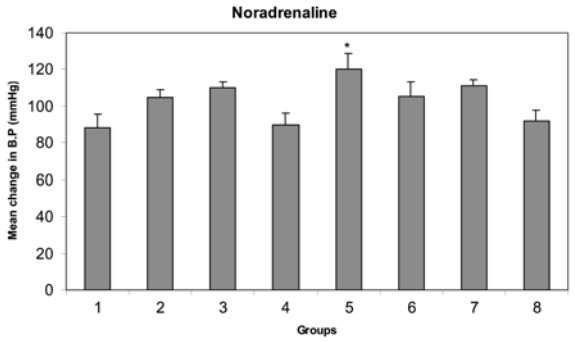

Fig. 3. Mean change in blood pressure to noradrenaline (0.5 g/kg) in (1)-Sham control, (2)-PE-50, (3)-TF-10, (4)-KGE-30, (5)-DOCA, (6)-DOCA+PE-50, (7)-DOCA +TF-10, (8)-DOCA + KGE-30 treated groups. $^{*}P < 0.05$ when compared to sham control. Vertical lines represent S.E.M. (n = 5). B.P: Blood Pressure.

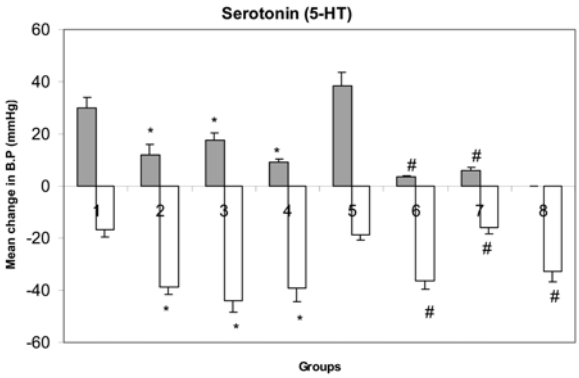

Fig. 4. Mean change in blood pressure to 5-HT (0.5 g/kg) in (1)-Sham control, (2)-PE-50, (3)-TF-10, (4)-KGE- 30, (5)-DOCA, (6)-DOCA+PE-50, (7)-DOCA+TF-10, (8)-DOCA + KGE-30 treated groups. $^{*}P < 0.05$ when compared to sham control and $^{\#}P < 0.05$ when compared to DOCA hypertensive rats. Vertical lines represent S.E.M. (n = 5). B.P: Blood Pressure; 5-HT: 5-Hydroxytryptamine.

Effect of PE-50 and KGE-30 on vascular reactivity to 5-HT, PBG and ACh in fructose hypertensive rats

Pressor responses to 5-HT was altered significantly ($P < 0.05$) in case of fructose (10%) fed hypertensive rats as compared to control rats. Pressor responses to 5-HT were significantly reduced ($P < 0.05$) in case of fructose (10%) fed rats, which received PE-50

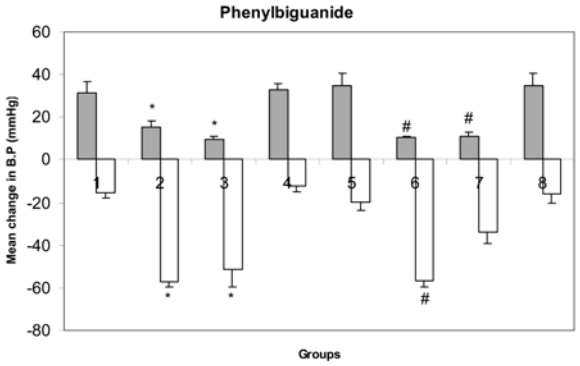

Fig. 5. Mean change in blood pressure to phenylbiguanide (PBG - 0.5 g/kg) in (1)-Sham control, (2)-PE-50, (3)-TF-10, (4)-KGE- 30, (5)-DOCA, (6)-DOCA+PE-50, (7)-DOCA+ TF-10, (8)-DOCA+KGE-30 treated groups. $^{*}P < 0.05$ when compared to sham control and $^{\#}P < 0.05$ when compared to DOCA hypertensive rats. Vertical lines represent S.E.M. (n = 5). B.P: Blood Pressure.

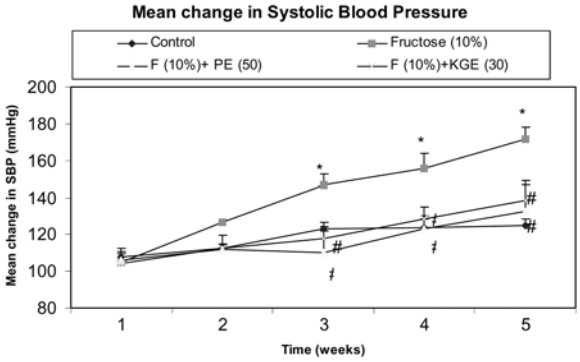

Fig. 6. Time course (weekly) of changes in mean arterial pressure (mm Hg) during 5 w in Control, Fructose (10%), F (10%) +PE-50, F (10%) + KGE- 30 treated groups. $^{*}P < 0.05$ when compared to control group. $^{\#}P < 0.05$ when compared to F (10%) rats. Vertical lines represent S.E.M. (n = 5).

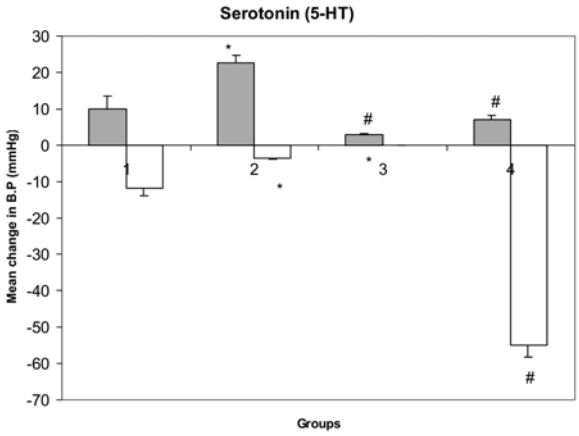

Fig. 7. Mean change in blood pressure to 5-HT (0.5 g/kg) in (1)-control, (2)-Fructose (10%), (3)-F (10%) +PE-50, (4)-F (10%) + KGE-30 treated groups. $^{*}P < 0.05$ when compared to control group. $^{\#}P < 0.05$ when compared to F (10%) rats. Vertical lines represent S.E.M. (n = 5). B.P: Blood pressure; 5-HT: 5-Hydroxytryptamine.

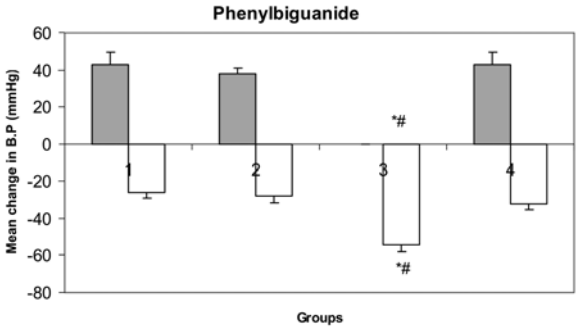

Fig. 8. Mean change in blood pressure to phenylbiguanide (PBG - 0.5 g/kg) in (1)-control, (2)-Fructose (10%), (3)-F (10%) + PE-50, (4)-F (10%) + KGE-30 treated groups. $^{*}P < 0.05$ when compared to control group. $^{\#}P < 0.05$ when compared to F (10%) rats. Vertical lines represent S.E.M. (n = 5). B.P: Blood Pressure.
■ = Rise in blood pressure, □ = Fall in blood pressure.

and KGE-30 for 5 weeks as compared to fructose (10%) fed hypertensive rats. Pressor responses to PBG were significantly reduced ($P < 0.05$) in case of fructose (10%) fed rats, which received PE-50 for 5 w as compared to fructose (10%) fed hypertensive rats. Depressor responses to Ach were significantly increased ($P < 0.05$) in case of fructose (10%) fed rats, which received KGE-30 for 5 w as compared to fructose (10%) fed hypertensive rats (Figs. 7-9).

Effect of PE-50 and KGE-30 on Dose Response Curve of 5-HT on isolated rat stomach fundus strip of control and fructose hypertensive rats

Chronic administration of PE-50 and KGE-30 for 5 weeks in fructose hypertensive rats shifted dose response curve of 5-HT significantly ($P < 0.05$) to right with suppression of maxima as compared to dose response curve of fructose hypertensive rats on isolated stomach fundus strip (Fig. 10).

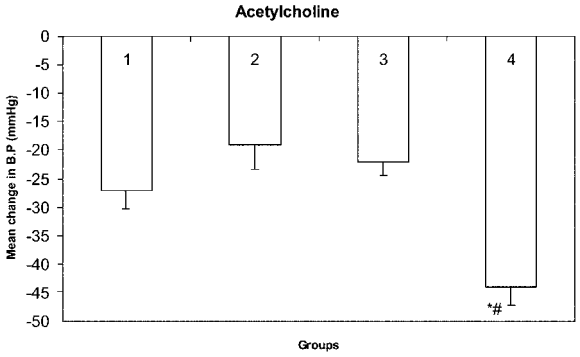

Fig. 9. Mean change in blood pressure to acetylcholine (Ach - 0.5 g/kg) in (1)-control, (2)-Fructose (10%), (3)-F (10%) + PE-50, (4)-F (10%) + KGE- 30 treated groups. $^{*}P < 0.05$ when compared to control group. $^{\#}P < 0.05$ when compared to F (10%) rats. Vertical lines represent S.E.M. (n = 5). B.P: Blood Pressure.

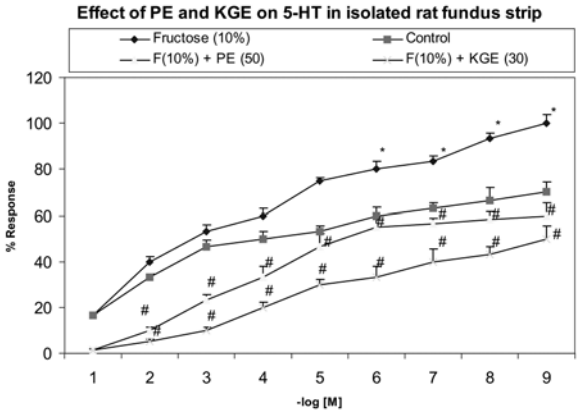

Fig. 10. Effect of PE-50 and KGE-30 on CDRC of 5-HT on isolated rat fundus strip in Control, Fructose (10%), F (10%) + PE-50, F (10%) + KGE-30 treated groups. $^{*}P < 0.05$ when compared to control group. $^{\#}P < 0.05$ when compared to F (10%) rats. Vertical lines represent S.E.M., n = 5. 5-HT = Serotonin (5-hydroxytryptamine).

Gas chromatography mass spectroscopy

The GC-MS showed presence of [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shagoal, [8]-shagoal [10]-shagoal and Zingiberone having molecular weights 294, 332, 360, 276, 306, 332 and 194 respectively. They contributed to 13.31%, 0.73%, 0.87%, 26.39%, 15.87%, 0.73%, and 1.51% respectively (Maryadele *et al*., 2001).

DISCUSSION

Zingiber officinale is well known for its antiemetic effects (Bone *et al*., 1990; Fischer *et al*., 1991), which is mediated through its 5-HT_3 antagonistic properties. This made us explore the antihypertensive effects of this plant through its 5-HT_3 antagonistic effects using phenylbiguanide. Literature survey has indicated that the aqueous ginger extract and its phenolic constituents lowers BP through a dual inhibitory effect mediated via stimulation of muscarinic receptors and blockade of Ca^{2+} channels (Ghayur *et al*., 2005; Ghayur and Gilani, 2005). Ginseng is also being used as one of the commonly used over the counter herbal prescription in patients with cardiovascular disease (Pharand *et al*., 2003). Korean red ginseng lowers blood pressure and can improve the vascular endothelial dysfunction in patients with hypertension possibly through increasing the synthesis and release of nitric oxide (Han *et al*., 1998; Jeon *et al*., 2000; Sung *et al*., 2000). Although different mechanisms for the antihypertensive effects of these plants have been reported, no mechanistic studies involving the 5-HT have been hypothesised. In search for better alternatives to antihypertensive drugs, the 5-HT_3 receptor antagonists, and 5-HT_{2B} antagonists are currently being considered for their potential use in hypertension (Tsukomoto *et al*., 2000; Shingala and Balaraman, 2005). Thus, this prompted us to direct our hypothesis highlighting the involvement of 5-HT in mediating the antihypertensive effects of the plants using DOCA salt and Fructose models of hypertension in rats.

The aim of the present study was to investigate the antihypertensive property of pet ether extract (PE) and toluene fraction (TF) of pet ether extract of *Zingiber officinale;* and *Korean ginseng* extract (KGE) in DOCA salt and fructose hypertensive rats. The study showed that chronic administration of PE (50 mg/kg/day; p.o), TF (10 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o.) for four weeks significantly reduced blood pressure in unilateral nephrectomized DOCA salt hypertensive rats. Chronic administration

of PE (50 mg/kg/day; p.o.) and KGE (30 mg/kg/day; p.o.) for five weeks significantly reduced blood pressure in fructose hypertensive rats.

DOCA-salt being a mineralocorticoid causes salt and water retention and by this way contributes to the development of hypertension. Endothelin-1 (Matsumura *et al*., 1999), atrial natriuretic peptides (Ogawa *et al*., 1999), vasopressin (Bereck *et al*., 1982) and 5-hydroxytryptamine (Dawson *et al*., 1988) are involved in the pathogenesis of this type of hypertension. Female rats were used in the DOCA salt hypertensive model as they appear to be more susceptible to develop hypertension (Greenberg *et al*., 1973; Balaraman *et al*., 1989).

The 5 HT_{2B} receptor, first called atypical 5-HT receptor (Vane, 1957) is a highly sensitive 5-HT receptor in the longitudinal smooth muscle of the rat stomach fundus. Recently it has been hypothesized that in hypertension there is upregulation of 5-HT_{2B} receptor inorder to maintain an elevated blood pressure in rats made hypertensive by DOCA and NAME (Banes and Watts, 2002; Russel *et al*., 2002). It has been previously reported that part of this increase in arterial sensitivity to 5-HT is due to a change in the receptor population that mediates contraction to 5-HT under conditions of DOCA-salt hypertension. 5-HT possesses 300-1,000 times higher affinity for the 5-HT_{2B} receptor compared with the 5-HT_{2A} receptor (Wainscott *et al*., 1996) thus lower concentrations of 5-HT are necessary to activate the 5-HT_{2B} receptor.

Bilateral microinjection of 5-HT_3 receptor agonist, Phenylbiguanide (1.7 - 5 nmol) in nucleus tractus solitarius produced an increase in blood pressure and reduced the cardiovagal component of the baroreflex (Marahi and Laguzzi, 1995). The initial depressor response to Phenylbiguanide (PBG) is due to stimulation of known pulmonary chemoreceptors (including J receptors) whereas the pressor effect of phenylbiguanide is due to activation of receptors in the proximal arterial circulation (Giles and Sander., 1986).

On the basis of these findings we wished to test the putative 5-HT_{2B}/5-HT_3 antagonistic property of PE, TF or KGE in DOCA-salt hypertensive and fructose models. PE (50 mg/kg/day; p.o); TF (10 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o) were able to reduce blood pressure in DOCA-salt hypertensive rats but did not alter blood pressure in sham operated control rats implying an antihypertensive effect. Effects of PE and TF on vascular reactivity support that these act through influencing serotonergic pathway in hypertensive rats, as those selectively blocked the rise of 5-HT and PBG in hypertensive rats, which is mediated by 5-HT_2 and 5-HT_3 receptors whereas KGE selectively blocked the rise of 5-HT in DOCA model. Further, antihypertensive effect of PE, TF and KGE does not involve modulation of the renin-angiotensin system since the antihypertensive effect was observed in DOCA-salt hypertensive rats, a low renin model of experimental hypertension.

Recent studies have shown that a high fructose diet is associated with increased blood pressure in rats (Bunnag *et al*., 1997; Dimo *et al*., 2001a, 2001b; Juan *et al*., 1988). An impaired response to endothelium-dependent vasodilators in fructose fed rats has also been demonstrated (Richey *et al*., 1998). A growing body of evidence indicates that locally generated vasoactive substances such as angiotensin-II (Ang-II) and nitric oxide (NO) are important determinants of the natural history of vascular disease. Recent evidence suggests that endothelial NO production could be decreased in fructose fed rats at both renal (Nagai *et al*., 2002) and vascular levels (Shinozaki *et al*., 1999). Alterations in both endothelial production of NO and VSMC growth could be associated with the initiation or progression of the atherosclerotic process and to vascular changes in hypertension. Korean red ginseng can improve the vascular endothelial dysfunction in patients with hypertension possibly through increasing the synthesis of nitric oxide (Jeon *et al*., 2000; Sung *et al*., 2000).

In the present study, the effect PE (50 mg/kg/day; p.o.) and KGE (30 mg/kg/day; p.o.) were examined on hypertension induced by fructose (10%) given

in drinking water. Male rats were used in this study as female rats do not develop hypertension or hyperinsulinemia upon fructose feeding except after ovirectomy, suggesting that female sex hormones may confer protection against the effects of a fructose diet (Galipeau *et al*., 2002). Parameters like Systolic blood pressure and vascular reactivity to 5-HT, PBG were modified significantly by chronic administration of PE (50 mg/kg/day; p.o.) and KGE (30 mg/kg/day; p.o) in fructose hypertensive rats. Moreover vascular reactivity to ACh was significantly altered by chronic administration of KGE (30 mg/kg/day; p.o) in fructose hypertensive rats.

Results from vascular reactivity also support the serotonin antagonistic property of PE and KGE. The inhibition of pressor effect due to 5-HT and PBG in hypertensive rats, which is due to 5-HT_{2B} and 5-HT_3 receptor mediated respectively gives a clue that PE may act through 5-HT_{2B} and 5-HT_3 receptors. The inhibition of pressor effect due to 5-HT in both the models supports the possible 5-HT_{2B} antagonistic activity of KGE. The significant potentiation of depressor response of ACh in fructose model by KGE may be possibly due to the NO production property of KGE.

The sensitivity of rat fundus to 5-HT from fructose induced hypertensive rats was increased as compared to that in control rats. Treatment with PE (50 mg/kg/day; p.o.) and KGE (30 mg/kg/day; p.o.) in fructose hypertensive rats for 5 weeks shifted the cumulative dose response curve of 5-HT significantly to the right compared to fructose hypertensive rats suggesting upregulated 5-HT_{2B} receptors may be blocked. The GCMS data indicates the presence of gingerols (nearly 15%) - a 5-HT_3 antagonist component in ginger which further supports the data.

Collectively, the results of our study suggest that 5-HT plays an important role in development of hypertension and the antihypertensive activity of *Zingiber officinale* may be due to 5-HT_{2B}/5-HT_3 receptor antagonism and *Korean ginseng* extract may be due to 5-HT_{2B} antagonism and NO production although the validity of the earlier mentioned mechanisms such as calcium blocking effects or stimulation of muscarinic receptors for antihypertensive effects cannot be questioned.

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